

# The Isomerization Kinetics of Lactose to Lactulose in the Presence of Borate

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**Abstract:** The kinetics of the isomerization of lactose to lactulose in the presence of boric acid and NaOH was studied. A first order equation fits the data for the rate controlling reaction—the formation of a boric acid–lactulose complex. Since the reaction is optimum at a molar ratio of boric acid to lactose of 1.0 and pH 11, a model was developed for application at these reaction conditions.

**Key words:** reaction model, lactulose, boric acid.

## NOTATION

$E$	Activation energy gas constant (K)
$GAL$	Galactose, galactose concentration
$HB$	Boric acid, boric acid concentration
$HL$	Boric acid/lactulose complex
$ILS$	Lactulose, lactulose concentration
$ILS_{eq}$	Equilibrium lactulose concentration
$k_0$	Frequency factor ( $\text{min}^{-1}$ )
$k_1$	Rate constant for disappearance of lactose ( $\text{min}^{-1}$ )
$k_2$	Rate constant for formation of lactulose ( $\text{min}^{-1}$ )
$L$	Lactose, lactose concentration
$L_0$	Initial lactose concentration
$t$	Time (min)

## 1 INTRODUCTION

Isomerization of reducing sugars is a well known reaction in carbohydrate chemistry. First discovered by Lobry de Bruyn and van Ekenstein,<sup>1</sup> the reaction has been used to prepare unavailable carbohydrates by isomerization or epimerization of naturally occurring carbohydrates.

Lactulose is a complex carbohydrate, highly valued as

a pharmaceutical with world-wide markets. Lactulose is normally synthesized by isomerization of lactose. Recent methods for producing lactulose involve the use of complexation reagents such as aluminate<sup>2,3</sup> and borate.<sup>4,5</sup> These compounds shift the equilibrium established during base catalyzed isomerization in favor of lactulose and prevent degradative side-reactions. These reagents permit formation of lactulose in high yields, but both are impractical since aluminate<sup>2</sup> is difficult to remove and a huge excess of borate<sup>4</sup> is necessary for optimal yield.

Hicks and colleagues<sup>5,6</sup> showed that treatment of lactose with boric acid in a molar ratio of 1.0 in the presence of tertiary amines produces lactulose in high yields. The process allows for recovery and recycling of reagents; a technique which increases the efficiency of the process.

More recently, Hicks *et al.*<sup>6,7</sup> showed that NaOH can be substituted for the tertiary amine. In a further study, Hicks *et al.*<sup>8</sup> showed the boric acid catalyst could be removed and recycled using a commercially available polystyrene–divinylbenzene resin, which contains (covalently attached) 1-deoxy-1-methylamino-D-glycitol functional groups.

A study has begun to develop a commercially feasible process to produce lactulose based upon the use of the borate ion and NaOH to isomerize lactose. The first step in this study was the development of an engineering model for the kinetics of the isomerization reaction. This paper presents the results of that study.

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† Reference to brand or firm names does not constitute an endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

## 2 EXPERIMENTAL

Reactions were performed in jacketed glass reactors. Lactose and boric acid (HB) were weighed into the reactor and deionized water added. A typical experiment used 20 g lactose, 3.65 g boric acid, 100 cm<sup>3</sup> water, and 50% NaOH to adjust the pH to the desired level. The lactose and HB dissolved when the pH reached about 9.

Temperature was controlled with a constant temperature water-bath circulating water through the jacket of the reactor. The reactor was fitted with a rubber stopper, thermocouple, and magnetic stirrer. Reaction time started when the controlled temperature water began circulating through the jacket of the reactor. It usually took about 12 min for the reaction mixture to reach the temperature of the controlled water.

Samples were taken at intervals determined appropriate for the particular experiment. Intervals ranged from 5 min to 1 h. Nominal 5 cm<sup>3</sup> samples were taken and placed in an ice/water bath to quench the reaction.

The carbohydrate composition was determined using HPLC with refractive index detection. A Bio-Rad HPX-87 H, Aminex ion exclusion column was used. The conditions for this analysis are:

Mobile phase = 0.013 M nitric acid  
Column = 300 mm × 7.8 mm  
Column temperature = ambient  
Flow rate = 1.0 cm<sup>3</sup> min<sup>-1</sup>  
Refractive index detector  
Sample loop = 50 mm<sup>3</sup>

The HPLC system was calibrated before use with a mixed standard containing all of the components of interest at four concentration levels (0.2, 0.4, 0.8 and 1.0 mg cm<sup>-3</sup>). While the HPLC operating conditions used did not provide a baseline separation of lactose and lactulose, good accuracy over the concentration ranges studied was obtained using peak height measurements. Mixtures of lactulose and lactose at known concentrations were used for determination of accuracy. For lactose an error of ≤ 4% (average = 2.08%) was found over the range of 1.5 mg cm<sup>-3</sup> to 0.1 mg cm<sup>-3</sup> and for lactulose an error of ≤ 5% (average = 3.08%) was found over the range of 1.5 mg cm<sup>-3</sup> to 0.3 mg cm<sup>-3</sup> under these conditions. Samples were routinely diluted before HPLC analysis in order to obtain measurements within the most useful and accurate concentration range for the particular component.

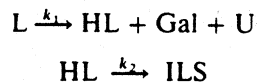
Lactose was edible grade purchased from Swiss Valley Farms Co., Davenport, IA (USA). The lactose was the monohydrate form. Results were corrected for water of hydration. Boric acid was NF granular purchased from US Borax. US Borax & Chemical Corp., Los Angeles, CA (USA).

## 3 RESULTS AND DISCUSSION

In the presence of base (NaOH) lactose isomerizes to lactulose in low yield with multiple side by-products. In the reaction system studied, boric acid complexes lactulose shifting the equilibrium in favor of lactulose. The complexation and shift in equilibrium also minimizes side products. When the reaction mixture is cooled and the pH lowered (acidified) the complex breaks releasing the product, lactulose.

The reaction, as understood by the authors, is as follows. Lactose apparently isomerizes initially to lactulose, most of which is instantly complexed by boric acid. It is well known<sup>9</sup> that lactulose breaks down in alkaline solutions into a mixture of galactose and α- and β-isosaccharinic acids. A small amount of the lactulose goes to these by-products instead of complexing. When the reaction mixture is acidified the complex instantly splits releasing lactulose and boric acid.

The isomerization of lactose to lactulose was modelled as two consecutive but separate reactions. The first reaction was considered the conversion of lactose to the boric acid complex of lactulose (HL) plus galactose (GAL) and other by-products (U). The second reaction was the disruption of this complex releasing lactulose (ILS).



According to Levenspiel<sup>10</sup> a linear plot of  $-\ln(L/L_0)$  vs time indicates a first order reaction model. A first order reaction model hypothesis was tested for the first reaction as shown in Fig. 1. The lactose concentration data plotted as a straight line indicating the use of a first order reaction model for the disappearance of lactose is suitable.

Assuming the formation of ILS is also first order then,

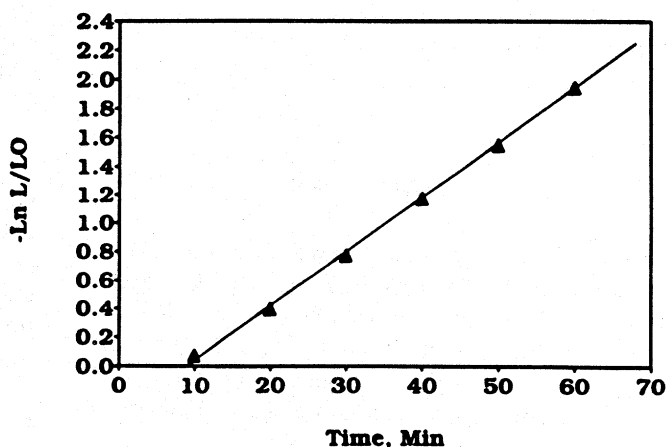


Fig. 1. Test for first order reaction model for reaction of lactose.  $L$  = Lactose concentration,  $L_0$  = initial lactose concentration; 20 wt%. Reaction conditions: 70°C, molar ratio  $H_3BO_3$ /lactose = 1.0, pH 11.

from Levenspiel,<sup>10</sup>

$$-dL/d\theta = k_1 L \quad (1)$$

$$-dHL/d\theta = dILS/d\theta = k_2 HL \quad (2)$$

The integrated form of eqn. (1) is:

$$L = L_0 \exp(-k_1 \theta) \quad (3)$$

The first step in developing a model is to determine the rate constant ( $k_1$ ) for the first reaction for a first order reaction model corresponding to eqn. (1). However, the time needed to reach isothermal conditions was about 10–12 min. This presented a small difficulty in determining  $k_1$  because the correlation is made using isothermal conditions and some of the experimental data were not isothermal. The data were fitted using plots like Fig. 1. The experimental data over the isothermal temperature range plotted as a straight line. Therefore, the rate constant was evaluated from the slope of the line as in Fig. 1 for the isothermal data. The equation was integrated over the isothermal portion only, from about 10 min to near equilibrium. A Hooke-Jeeves Pattern search was used to determine the best values of the rate constant,  $k_1$ , for each experiment. Experiments were made in the temperature range of 55 to 84 °C, a range of molar ratios of boric acid to initial lactose concentration,  $HB/L_0$  (0.5–3), and at several pH levels (pH = 10–12).

The next step in developing a model should be determination of rate constant  $k_2$  for eqn. (2). It was obvious working with this reaction system that the second reaction was not rate controlling. The complex is extremely stable at high pH. However, the conversion or disruption of the complex to lactulose is very quick at low pH. When the reaction mixture is acidified the complex breaks immediately to give lactulose. From a practical engineering standpoint only the first reaction needs a mathematical model. All that is needed for the second is a determination of the degree of conversion to lactulose based on the starting lactose; a value for  $k_2$  is not needed and was not determined. (In these studies the formation of lactulose from the breakdown of the complex took place in the HPLC during analysis. In the process under development, the pH is adjusted and the complex broken in a surge tank. It appears to be instantaneous or at least as fast as the pH equilibration reaction between HCl and NaOH.)

The rate controlling reaction for the model is the conversion of lactose to the complex. Assuming the concentration of lactulose after acidification equals the concentration of the complex before acidification, the equilibrium expression in eq. (4):

$$ILS = HL = L_0[1 - \exp(-k_1 \theta)]. \quad (4)$$

Initial attempts to fit the equation to the data were disappointing. The difficulty is that eqn. (4) assumes the total number of moles in the system is constant and equal to the initial number of moles of lactose,  $L_0$ . However,

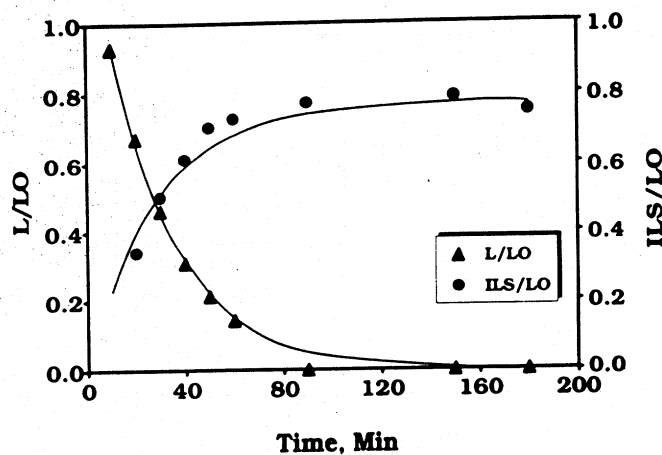


Fig. 2. Fit of the first order model to the conversion of lactose to lactulose.  $L$  = Lactose concentration,  $L_0$  = initial lactose concentration; 20 wt%,  $ILS$  = lactulose concentration. Reaction conditions; 70 °C, molar ratio  $H_3BO_3$ /lactose = 1.0, pH 11.

there is some destruction of lactose to unidentified components, e.g. caramelized carbohydrates which create the color. Assuming this destruction of lactose is due to competing first order reactions producing unwanted by-products, the equilibrium value of lactulose was substituted for  $L_0$  in eqn. (4):

$$ILS = ILS_{eq}[1 - \exp(-k_1 \theta)] \quad (5)$$

Although  $k_1$  is actually the sum of the rate constants of all competing reactions it was used as a practical approximation since the individual rate constants for each of the competing side reactions are unknown. The equilibrium value for lactulose used in eqn. (4) is not a true equilibrium value but rather a final concentration value due to reaction and partial destruction of lactose. The 'equilibrium' value used was taken as the value of lactulose realized in Figs 3 and 4 based on the specific reaction conditions.

For each experiment the first order kinetics model was used to evaluate the rate constant,  $k_1$ , using the Pattern search routine. Using the experimentally determined equilibrium value for lactulose formation plus the value determined for  $k_1$ , the model generally calculated the formation of lactulose quite well (Fig. 2). However, the correlation coefficient was sometimes lower, about 0.9, because of the poor fit for the first couple of data points before thermal equilibration.

Four variables remain to be investigated—temperature, pH, catalyst, and lactose concentrations. The effect of pH and catalyst was studied at 70 °C. In a series of experiments the rate constant,  $k_1$ , and equilibrium values,  $ILS/L_0$ , were determined as a function of molar ratio of boric acid to initial lactose concentration,  $HB/L_0$ . Figure 3 shows the effect of catalyst ratio on equilibrium conversion. The equilibrium increases rapidly with molar ratio until the ratio reaches about 1.0, then it gradually drops off. Within the range of pH 10.5 to 11.5, pH exerts little or no effect on the equilibrium conversion. At lower

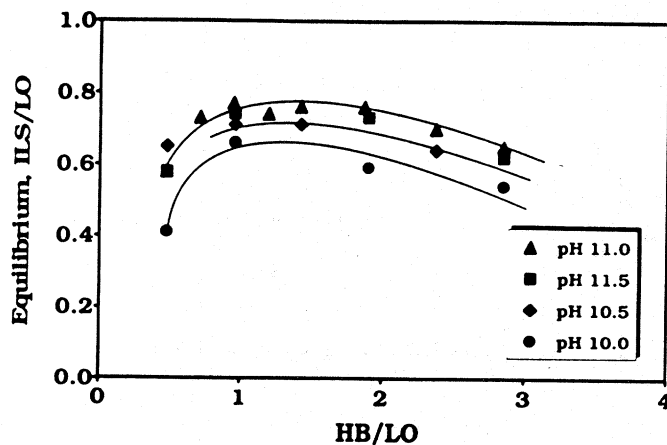


Fig. 3. Effect of molar ratio of catalyst to lactose on equilibrium value for lactulose at 70°C. *HB* = Boric acid concentration.  $L_0$  = initial lactose concentration; 20 wt%.

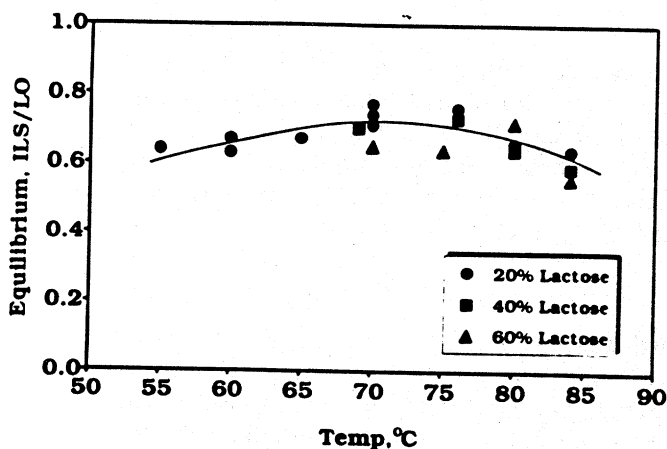


Fig. 4. Effect of temperature and lactose concentration on equilibrium value for lactulose: molar ratio  $H_3BO_3$  lactose = 1.0, pH 11.

pH levels the reaction simply does not proceed and at higher pH levels there is significant caramelization and difficulty in controlling the reaction. However, the maximum conversion occurs at a molar ratio, *HB*  $L_0$ , of 1.0 in the pH range of 10.5 to 11.5.

The effect of temperature and lactose concentration on equilibrium was studied (Fig. 4). There is a slight maximum in the range of 70–75°C. The optima for the borate to sugar molar ratio, reaction pH and temperature are consistent with that shown earlier for the conversion of lactose in cheese whey ultrafiltrate into lactulose, using the boric acid/NaOH reagent and for the conversion of lactose into lactulose when tertiary amines<sup>4</sup> and boric acid were used as reagents. There may also be a slight lowering of equilibrium as lactose concentration increases; however, the effect is small. Generally, these two variables exert only minor influence on equilibrium.

Figure 5 shows that, at pH 10.5 to 11.5, the rate constant,  $k_1$ , increases slightly with molar ratio, *HB*  $L_0$ . This is a very slight increase relative to the effect of

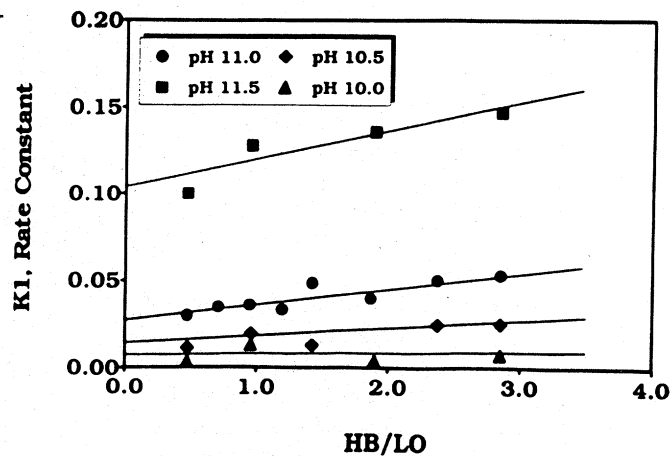


Fig. 5. Effect of molar ratio of boric acid to lactose on rate constant at various pH levels; 70°C. *HB* = Boric acid concentration.  $L_0$  = initial lactose concentration.

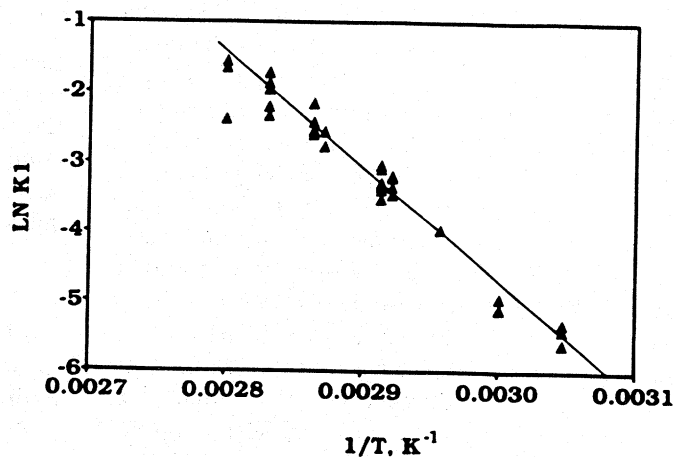


Fig. 6. Arrhenius plot at a molar ratio of 1.0 and pH 11.

temperature and pH on the rate constant. This does not represent enough of a rate increase to change the conclusion that the reaction should be run at a molar ratio of 1.0. At pH 10.0 the rate decreases slightly with the ratio. Figure 5 also shows that the value of  $k_1$  increases with pH.

The effect of temperature on the reaction rate was also studied. Rather than develop a model to cover all possible reaction configurations, a specific limited kinetics model, useful for engineering design, for temperature which applies at a molar ratio of 1.0 and a pH of 11 was developed. Figure 6 presents the Arrhenius plot at a molar ratio of 1.0 and pH 11. The correlation coefficient was 0.95. Equation (6) presents the kinetics model developed for the reaction of lactose to lactulose at pH 11 and a molar ratio of 1.0:

$$dL/dt = k_1 L \text{ where } k_1 = k_0 \exp(-E/RT) \quad (6)$$

and

$$k_0 = 8.4 \times 10^{18} \text{ min}^{-1}$$

$$E/R = 16095 \text{ K}$$

#### 4 CONCLUSIONS

A mathematical kinetics model was developed. This model is sufficiently accurate to design a continuous reactor system for a continuous process for processing lactulose from lactose. A first order kinetics model adequately describes the isomerization of lactose to lactulose in the presence of boric acid. The model uses two reactions—the first is the isomerization of lactose and complexation with boric acid and the second is the breaking of the complex and release of lactulose. The rate controlling reaction is the formation of a boric acid–lactulose complex. Optimum reaction conditions include a molar ratio of boric acid to lactose of 1.0 and a pH of 11. The equilibrium conversion of lactulose based on the initial concentration of lactose is about 75%.

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